AD_____

Award Number: W81XWH-06-1-0385

TITLE: FGFR4 Downregulation of Cell Adhesion in Prostate Cancer

PRINCIPAL INVESTIGATOR: Daniel J. Donoghue, Ph.D.
April N. Meyer
Kristine A. Drafahl

CONTRACTING ORGANIZATION: University of California
La Jolla, CA 92093-0934

REPORT DATE: March 2007

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01-03-2007	Annual	1 Mar 2006 – 28 Feb 2007

4. TITLE AND SUBTITLE

FGFR4 Downregulation of Cell Adhesion in Prostate Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0385

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Daniel J. Donoghue, Ph.D.; April N. Meyer and Kristine A. Drafahl

Email: ddonoghue@ucsd.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of California
La Jolla, CA 92093-0934

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
We have made excellent progress in the preliminary stages of our project to examine the role of FGFR4 G388R in altering cell adhesion in prostate cancer. This includes acquiring expertise in the passage and transfection for gene expression studies using prostate cancer cell lines. A key accomplishment is the demonstration of feasibility of ponasterone A inducibility of FGFR4 expression in PC3 cells. We hope to successfully create these inducible cells to use in our studies of FGFR4 G388R and its role in prostate cancer progression.

15. SUBJECT TERMS
FGFR4, FGFR4-PTD, PC3 cells, ecdysone inducible system, inducible expression

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	6	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

Introduction

Prostate cancer is currently the second most common type of cancer resulting in male mortality in the U.S. A recent publication demonstrates the importance of the common FGFR4 polymorphism Gly388Arg for the initiation and progression of prostate cancer. We set out to understand the role for this polymorphism in the proliferation and cellular adhesion of cells of prostatic origin. Previous investigations by our lab into differences between WT and G388R FGFR4 rendered few significant differences. This was possibly due to low transfection efficiencies, resulting in very small differences in endogenous signaling pathways. We set out to create prostate cancer cells inducible for FGFR4 expression to discover whether this method would make it easier to see slight differences in endogenous signaling. We were also interested in determining whether a truncated form of FGFR4 arising from alternative splicing, first identified in pituitary tumors, would increase the effects of the FGFR4 polymorphism.

Body

The first step in creating our FGFR4-inducible prostate cancer system was creating the pIND plasmids used for expression of the different FGFR4 protein products. Over the course of one month, pIND-FGFR4 plasmids were created by cutting the FGFR4 gene out of our current vector (pcDNA3) and ligating the fragments into the expression vector pIND provided with Invitrogen's ecdysone-inducible system.

These plasmids were then tested by transfecting 293-RXR cells with the pIND-FGFR4 plasmids and selecting for colonies over the course of one month. These colonies were expanded and tested for inducible expression of the FGFR4 protein products, which confirmed that the pIND-FGFR4 plasmids were functional (Figure 1).

In our proposal, one of the prostate cancer cell lines in which we chose to create our inducible system was the DU145 cell line. The first step in creating the inducible system required testing a range of antibiotic concentrations to determine the optimal concentration of antibiotics to select for incorporation of the expression vectors. After several weeks, we determined the optimal concentrations of antibiotics. Over the course of one month, these cells were first transected with the pVgRXR expression plasmid and selected with the antibiotic zeocin. Approximately 20 colonies were selected and expanded in the following weeks. We attempted to test these colonies using protocols suggested by Invitrogen. These included Western-blotting for the RXRalpha protein using a commercially available antibody. After several weeks of troubleshooting our attempts at detecting RXR and utilizing suggestions from the antibody manufacturer, we decided to test for RXR expression by transiently transfecting pIND-FGFR4 to test for inducibility. Again, we were unable to confirm that the components from the pVgRXR vector required for making the inducible system work had been incorporated into the cells.

For the next month, we decided to continue with the DU145 clones that had been selected with zeocin for RXR expression, without confirming that RXR had been incorporated. During this time, these cells were transfected with pIND-FGFR4 and selected with the G418 antibiotic. Colonies were selected, expanded and tested for inducibility by adding the inducer ponasterone A. After extensive testing of many clones, we found that no clones were inducible for FGFR4.

Invitrogen stated that some cell lines work well with their system, while other cell lines resist incorporating the correct components into their genome. We decided to try another type of prostate cancer cell line, PC3 cells, which have very low levels of endogenous FGFR4.

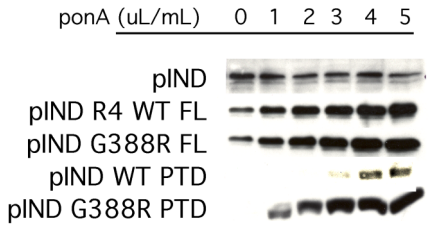
Again, cells were tested with an antibiotic killing curve over several weeks to determine the optimal concentrations of antibiotics. Next, PC3 cells were transfected with pVgRXR and selected with zeocin. Over the course of one month, approximately 20 colonies were selected and expanded. As with the DU145 cells, we attempted to determine whether RXR had indeed been incorporated into the genome by transiently transfecting pIND-FGFR4 and testing for inducibility. As a control, we also included a co-transfection of pIND-FGFR4 plus pVgRXR to confirm that the inducible system was working properly. Our transient test of inducibility showed no expression of FGFR4, indicating that the pVgRXR plasmid was not incorporated into the genome of PC3 cells (see an example blot in Figure 2).

We thought that it was possible the cells were somehow able to digest the plasmid and incorporate only the gene required for antibiotic resistance. This would explain why, under antibiotic selection, we were able to grow colonies of cells that were not expressing any of the pVgRXR components. Invitrogen suggested that by linearizing the plasmid, it may lessen the chances of the cells splicing the gene incorrectly. Therefore, we repeated the process using linearized pVgRXR. At the same time, we co-transfected another set of PC3 cells with both the pVgRXR and pIND-FGFR4 plasmid, and selected for colonies by treating with both zeocin and G418.

Currently, we are in the process of testing PC3 colonies that were transfected with linearized pVgRXR, and colonies that were co-transfected and co-selected for expression of pVgRXR plus pIND-FGFR4. We hope to determine whether either of these methods were successful in creating our inducible system in PC3 prostate cancer cells.

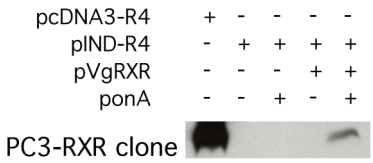
In the meantime, we have been conducting transient transfections in PC3 cells. Although this method is not optimal, we hope to obtain meaningful data while attempting to create the more desirable inducible cells. We have optimized transfection efficiency to allow for the highest transfection rate in order to increase the chances of detecting some significant difference between FGFR4 WT and FGFR4 G388R or between WT-PTD R4 and G388R-PTD R4.

Figure 1



We are examining basal receptor phosphorylation to determine whether the polymorphism increases the basal receptor activation. We are also looking at activation of downstream pathways such as MAPK, STATs, and AKT. Additionally, we are examining expression of cellular adhesion proteins such as N-cadherin, E-cadherin, P-cadherin, and NCAM to determine whether the G388R polymorphism allows for altered regulation of any of these proteins that are important for cellular motility.

Figure 2



Our hope is to successfully create FGFR4-inducible PC3 cells. This system should allow us to see any slight differences in activation of endogenous pathways due to differences in receptor activation. We also hope to determine whether the PTD truncation increases the activity of the G388R polymorphism. Understanding the role of G388R polymorphism in prostate cancer progression is extremely important, and we hope that our findings will help determine alternative therapies for the treatment of prostate cancer.

Key Research Accomplishments (as described more fully above)

- Construction of four different FGFR4 derivatives, as described in our proposal
- Successful demonstration of expression using the ecdysone inducible system
- Generation of zeocin/G418 “killing curves” for both DU145 and PC3 prostate cancer cells
- Detection of inducible expression of pIND-FGFR4 derivatives in 293-RXR cells
- Detection of inducible expression of pIND-FGFR4 derivatives in PC3 cells when pVgRXR and pIND-FGFR4 plasmids were introduced by transient transfection

Reportable Outcomes

N/A

Conclusion

We have made excellent progress in the preliminary stages of our project to examine the role of FGFR4 G388R in altering cell adhesion in prostate cancer. This includes acquiring expertise in the passage and transfection of prostate cancer cell lines for gene expression studies. A key accomplishment is the demonstration of feasibility of ponasterone A inducibility of FGFR4 expression in PC3 cells. We hope to successfully create these inducible cells to use in our studies of FGFR4 G388R and its role in prostate cancer progression.

References

N/A

Appendices

N/A